Supporting Documents

I. Experimental

Phage Farming

T1 and Φ11 bacteriophages were prepared by Plate lysis method[1]. A heavy suspension of bacteria from a 16 h incubated plate was suspended in 2 ml of Tryptic Soy Broth. Then 500 µl of bacterial suspensions, 500 µl of phage stock solution, and 200 µl of cold $CaCl_2$ at 4°C were added to a 15 ml falcon tube followed by adding 5ml of top agar (Tryptic soy broth containing 0.70% agar, cooled to 50°C) and mixing well, then pouring on prepared TSA plates (pre-warmed for 30 min at 37°C). The top agar was allowed to cool and the plates were incubated at room temperature overnight or until clear lyses of the whole plate were observed. The top agar was scrapped gently with a sterile spreader by adding 5-6 ml of 1X PBS. The scrapped top agar from all plates was poured into a 50 ml falcon tube, followed by centrifuging at 10,000 rpm for 10 min at RT. The supernatant was collected and pellet was discarded. The supernatant was filter-sterilized using a 0.45 µm syringe filter with 100 µl of phage filtrate being spread on a plain TSA plate and incubated overnight to ensure sterility.

Phage Purification and Concentration by PEG method

100 g of PEG (MW 10,000) and 6 g of NaCl was mixed with 250 ml of water, autoclaved, and pH adjusted to 7.2 under sterile conditions. A 1:4 volume ratio of PEG:Bacteriophage supernatant was prepared and refrigerated overnight (stable up to 2 weeks at 4°C), followed by centrifuging the tubes at 10,000 rpm for 2 h at 4°C. The supernatant was discarded and the tube was left in an inverted position for 10-20 min.

The pellet was resuspended in 0.1 of the original volume of phage suspension using PBS and stored at 4°C until needed.

Plaque formation assay for the phage attached surfaces

PTFE and PE surfaces containing covalently attached bacteriophages (T1 for Escherichia coli and Φ11 for *Staphylococcus aureus* sub spp RN4220) were used for plaque formation assay. In a typical experiment, overnight culture of bacteria (*E. coli* for T1 and Φ11 for *S. aureus*) were diluted at a 1:1,000 ratio in TSB and allowed to grow for 3 h. The cells were normalized up to 0.1 (OD^{600} nm). Two 15 ml falcon tubes containing 500 µL of respective bacteria were prepared. 5 ml of top agar (cooled to 50°C) was added to each tube and poured into thin layered TSA plates pre-warmed at 37°C for 30 min. Final buffer in which the surface was suspended and the surface without the phage attached to it as well as the phage itself were also included as negative and positive controls. The respective surfaces were then stabbed into the top agar before solidification. The plates were incubated at room temperature for 24-48 h and results were observed in the form of clear plagues seen around the surfaces. This experiment was performed independently for each type of surface attached with respective phages. For mixed T1:Φ11 phage attached surfaces, similar assays were performed. Three plates T1, Φ11, and T1/Φ11 for each phage attached surfaces were tested for plaque formation. Buffers in which the surfaces were suspended were also tested to eliminate any free bacteriophages present. Positive and negative controls were included separately in each assay. Images were taken to record the plaques produced by the phages.

Phage attachment

Medical grade PTFE and UHMWPE (PE) were purchased from McMaster-Carr Supply Co. (Atlanta, GA), cut into 1 x 1 cm squares, washed in isopropanol, and dried at room temperature under vacuum before use. To obtain –COOH terminated PTFE and PE surfaces, microwave plasma reactions were conducted in the presence of maleic anhydride (MA) (Aldrich Chemical Co.) under open reactor conditions, as described elsewhere.[2,3] In the next step, PTFE-COOH and PE-COOH surfaces were placed in PBS buffer pH 7.4 (Invitrogen) containing 2.5 mmol of EDAC and 2.5 mmol NHS for 2 h in order to create $–COO^-$ groups followed by washing in PBS buffer, then immediate immersion into 10 mL buffer solution containing 500 µL of concentrated T1 phage or Φ11 phage from above for 16 h. Additional PTFE and PE surfaces were reacted with a mixture of T1 and Φ11 phage following the aforementioned process using 500 µL of each phage in 10 mL of PBS buffer. The surfaces were then washed seven times in PBS buffer to remove all non-covalently attached phages.

II. Analysis

Attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectra were collected using a Bio-Rad FTS-6000 FT-IR single-beam spectrometer set at a 4 cm^{-1} resolution equipped with DTGS detector and a 45° face angle Ge crystal with a depth of penetration of 0.37 μm. Each spectrum represents 200 co-added scans ratioed against a reference spectrum obtained by recording 200 co-added scans of an empty ATR cell. All spectra were corrected spectral distortions using Q-ATR software.[4]

Atomic force microscopy (AFM) measurements were conducted on a Bruker Dimension icon scanning probe microscope with ScanAssist, Digital Instruments. A silicon probe with 125 μm long silicon cantilever, nominal force constant of 40 N/m and resonance frequency of 275 kHz were used in a ScanAssist Air tapping mode, allowing assessment of surface topography. Quantification of bacteriophages covalently attached to Si surfaces was performed by using ImageJ software (NIH)[5] to analyze surface particles. The AFM images shown in Figure 4 of the Main Document represent randomly selected 5 x 5 μm areas from ~1 x 1 cm^2 polymer specimens utilized in these studies (the actual size of each specimen is illustrated in Figure 5 photographs).

III. Results and Discussion

Figure S-1 illustrates ATR-FTIR spectra recorded from Si surfaces. While Traces A shows the spectra for the Si wafer surfaces, Traces B represents the spectra of maleic anhydride plasma modified Si-MA surfaces. When T1 or Φ11 phages are reacted to Si-MA, the spectra shown in Traces C are obtained. As seen, two characteristic bands at 1653 and 1543 cm^{-1} due to Amide I and II bands characteristics of the T1 or Φ11 outer functionalities are detected, which are due to covalent attachment of bacteriophage to acid-functionalized Si surfaces. For comparison, Traces D in Figure S-1, (a) and (b) illustrate ATR-FTIR spectra of T1 or Φ11, respectively.

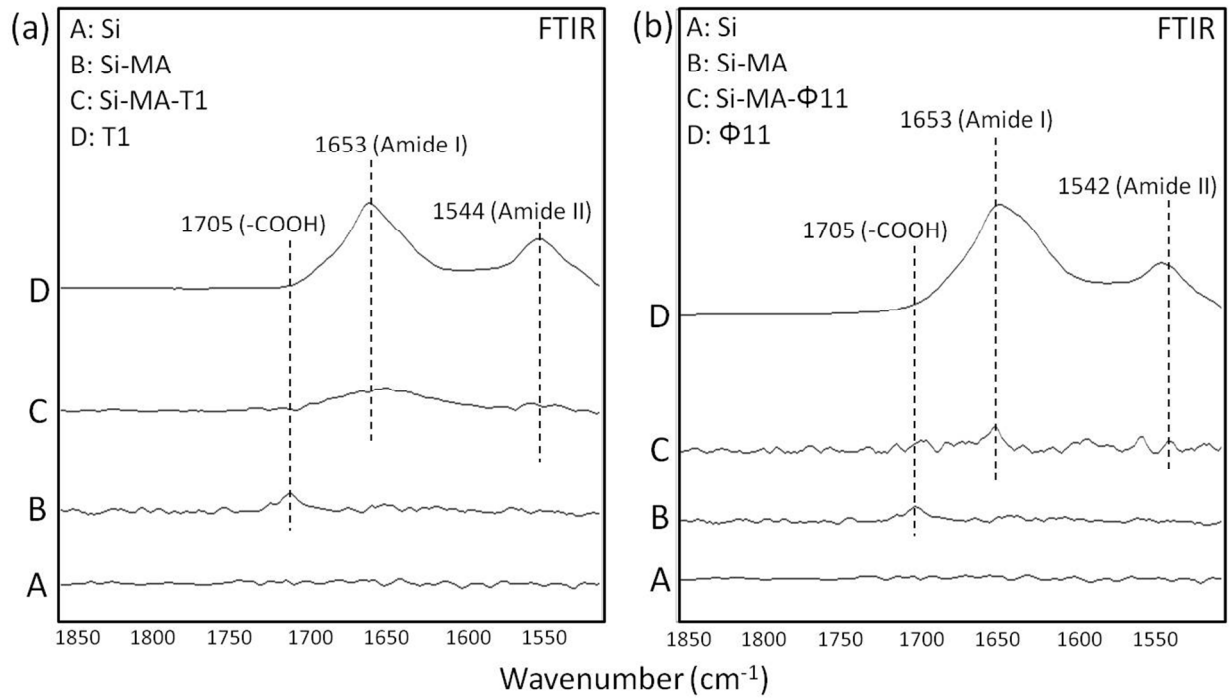

Figure S-1. A: ATR-FTIR spectra of Si surfaces; B: after plasma reactions on Si surfaces in the presence of maleic anhydride (Si-MA); C: after T1 (a) or Φ11 (b) phage

covalentl attachment to Si-MA surfaces (Si-MA-T1 and Si-MA- Φ11); D: Reference spectra of T1 (a) or Φ11 (b) (at 20% scale).

Biological activity of phages covalently attached to Si surfaces (Figure S-1) was confirmed using plaque formation assays of T1 and Φ11 phages. The results of exposure to their respective bacterial hosts *E. coli* and *S. aureus* are shown in Figure S-2, A and B. As manifested by the clear zone around each substrate, covalently attached T1 phages (Si-MA-T1) kill *E. coli* bacteria (Figure S-2, A), while Si-MA-Φ11 phages kill *S. aureus* bacteria (Firgure S-2, B), and the effectiveness of T1 and Φ11 phages covalently attached to Si surfaces is apparent.

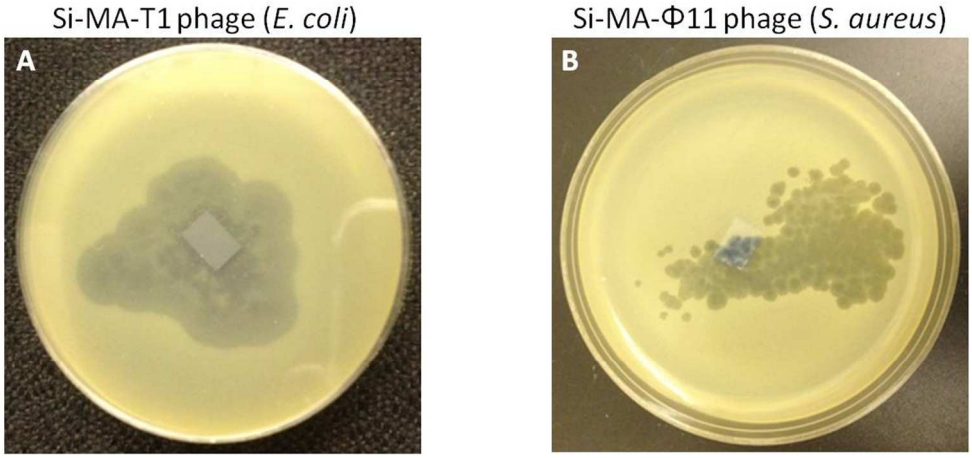

Figure S-2. Plaque formation assays on Si-MA-T1 (A) and Si-MA-Φ11 (B) wafer substrates upon exposure to *E. coli* (A) and *S. aureus* (B) .

In contract to PE-MA-T1 and PTFE-MA-Φ11 (main document) and Si-MA-T1 and Si-MA-Φ11 (Figure S-2), Figures S-3 and S-4 illustrate that biological activity of PE, PTFE, and Si (Figure S-3) as well as PE-MA, PTFE-MA, and Si-MA (Figure S-4) without the covalent attachment of T1 and Φ11 phages against *E. coli* and *S. aureus* does not occur. As shown, the abundant growth of both *E. coli* and *S. aureus* bacteria on the plaque formation assays occurs, thus not inhibiting bacterial growth.

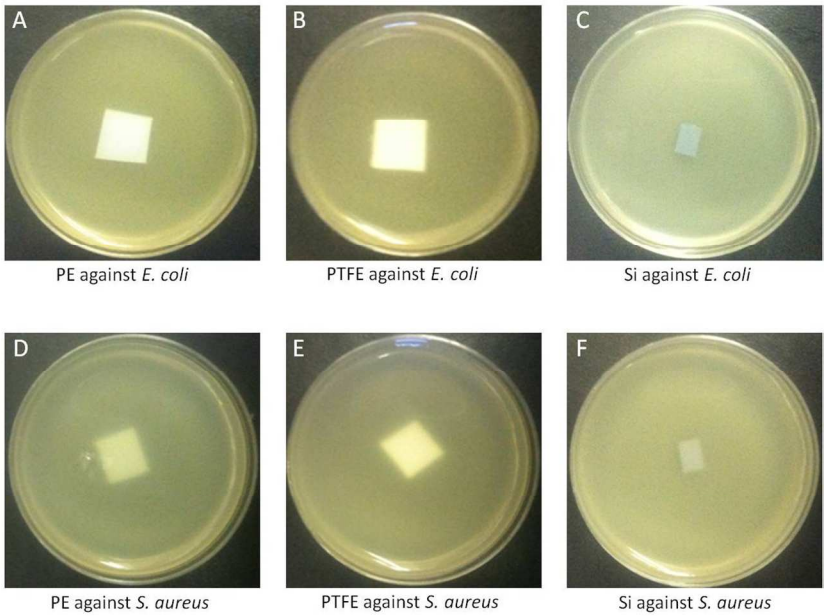

Figure S-3. Plaque formation assays against *E. coli* on PE (A), PTFE (B), Si (C) controls and against *S. aureus* on PE (D), PTFE (E), and Si (F) controls.

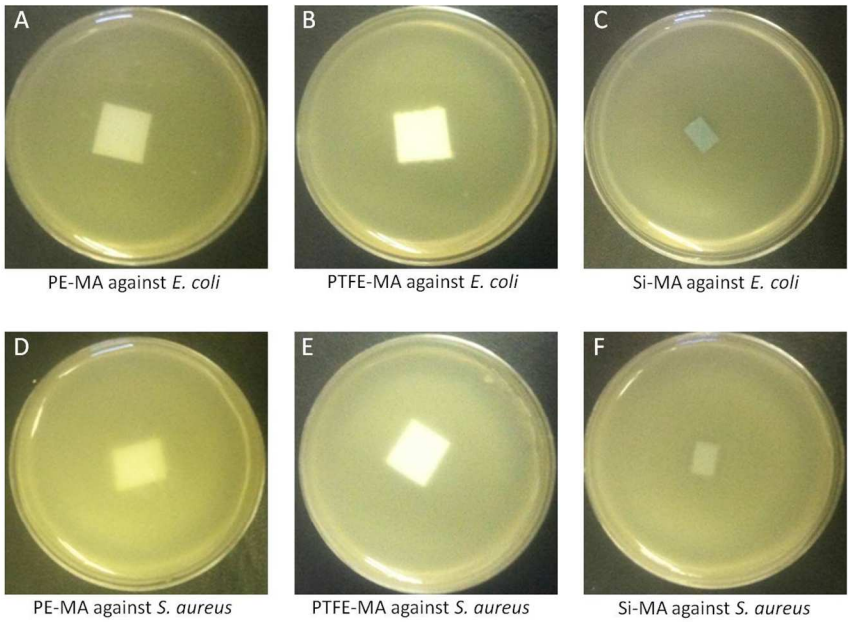

Figure S-4. Plaque formation assays against *E. coli* for plasma reacted maleic anhydride PE-MA (A), PTFE-MA (B), and Si-MA (C) substrates and against *S. aureus* bacteria plasma reacted maleic anhydride on PE-MA (D), PTFE-MA (E), and Si-MA (F) controls.

IV. References

1. Sambrook, J.; Russell, D. W., *Molecular Cloning: A laboratory manual* 3rd ed.; Cold Spring Laboratory press: Cold spring Harbor, New York, 2001; Vol. 1.

2. Gaboury, S. R.; Urban, M. W., Microwave plasma reactions of solid monomers with silicone elastomer surfaces: a spectroscopic study. *Langmuir* **1993,** 9, 3225.

3. Aumsuwan, N.; Heinhorst, S.; Urban, M. W., Antibacterial surfaces on expanded polytetrafluoroethylene; penicillin attachment. *Biomacromolecules* **2007,** 8, 713.

4. Urban, M. W. In *ATR Spectroscopy of Polymers-Theory and Practice*, ACS, Washington, D.C., 1996; Washington, D.C., 1996.

5. ImageJ Software. *National Institutes of Health*. http://rsb.info.nih.gov/ij/.